Paper No. 20

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte RICHARD J. YOULE, VEENA M. VASANDANI
YON-NENG WU, ESTER BOIX, and WOJCIECH ARDELT

Appeal No. 2001-0733
Application No. 09/095,429

ON BRIEF

Before WINTERS, WILLIAM F. SMITH, and GREEN, Administrative Patent Judges.

GREEN, Administrative Patent Judge.

DECISION ON APPEAL

This is a decision on appeal under 35 U.S.C. § 134 from the examiner's final rejection of claims 15-22. We note that this appeal is related to Appeal No. 02-0425, USSN 08/626,288, which is being decided concurrently with the instant appeal. Claim 15 is representative of the subject matter on the instant appeal, and reads as follows:

15. An isolated nucleic acid molecule encoding a recombinant ribonuclease from Rana pipiens oocytes (rOnc protein), comprising:

a polypeptide of SEQ ID NO: 1 having a glutamine residue at position 1, a hydrophobic residue other than methionine at position 23, a lysine at position 9, a histidine at position 10, a histidine at position 97, a lysine at position 31, a phenylalanine at position 98, and a threonine at position 35, and no more than a single conservative amino acid substitution at other than positions 1, 9, 10, 23, 31, 35 and 98, wherein the conservative amino acid substitution does not introduce a methionine into the polypeptide; and

a methionine at position -1 that can be subsequently cleaved to allow cyclization of the glutamine residue at position 1 to form a pyroglutamyl residue, such that the resulting protein is ribonucleolytic and cytotoxic.

Claims 17-19 are drawn to a method of making the polypeptide by expressing the claimed nucleic acid. Claim 20 is drawn to a host cell that expresses the nucleic acid molecule of claim 15, and claims 21 and 22 are drawn to expression vectors. Claims 16, 18 and 22 specify that the encoded for polypeptide has a leucine at position 23.

The examiner relies upon the following references:

Ardelt et al. (Ardelt) "Amino Acid Sequence of an Anti-Tumor Protein from Rana Pipiens Oocytes and Early Embryos," The Journal of Biological Chem. Vol. 266, No.1 pp. 245-251 (1991)

Mosimann et al. (Mosimann) "Refined 1.7 Å X-ray crystallographic structure of P-30 protein, an amphibian ribonuclease with anti-tumor activity," J. Mol. Biol Vol. 236 pp.1141-1153 (1994)

Creighton "Protein: Structure and Molecular Properties," second edition, W.H. Freeman and Company, New York pp. 9, 62 and 63 (1993)

Rybak et al. (Rybak) "Cytotoxic Onconase and Ribonuclease A Chimeras: Comparision and in Vitro Characterization," Drug Delivery, Vol. 1, pp. 3 –10 (1993)

Louis et al. (Louis) "Autoprocessing of the HIV-1 Protease Using Purified Wild-Type and Mutated Fusion Proteins Expressed at High Levels in Esherichia Coli," Eur. J. Biochem, Vol. 199 pp. 361-369 (1991)

Claims 15-22, all of the claims pending, stand rejected under 35 U.S.C. § 103(a) over the combination of Ardelt or Mosimann and Rybak, and over the state of the art as exemplified by Creighton and Louis. After careful review of the record and consideration of the issues before us, we reverse.

DISCUSSION

Claims 15-22 stand rejected under 35 U.S.C. § 103(a) as being rendered obvious by the combination of Ardelt or Mosimann and Rybak, in view of the state of the art as exemplified by Creighton and Louis.

Ardelt and Mosimann are cited by the rejection for teaching the amino acid sequence of onconase, which sequence is identical to SEQ ID NO: 1. Ardelt, according to the rejection, also teaches that cyanogens bromide treatment of the native protein cleaves the protein into two fragments. See Examiner's Answer, page 8. Rybak is cited for teaching that oncanase is currently in clinical trials for the treatment of cancer. See id. at 10.

Mosimann is also cited by the examiner for teaching the three-dimensional structure of onconase. According to the rejection,

> [t]he structure shows that the active site of the enzyme is formed from the amino acid of Pyr-1 (pyroglutamic acid), Lys-9, His-10, Lys-31, Thr-35, His-97 and Phe-98, see page 1149, left column, second paragraph. Thus, any onconase missing any of these important amino acids is expected to have compromised enzymatic and the associated cytotoxic activity.

Id. at 9.

The rejection concludes:

> Rybak [ ] disclose[s] that onconase is in clinical trial for the treatment of cancer, see page 4, first paragraph, which is motivation to one of ordinary skill in the art to make large quantities of onconase. Since the proteolytic processing of the methionine residue at the N-terminus of eukaryotic protein expressed in E. Coli is a known problem in the art, the amino acid sequence of SEQ ID NO: 1 has only one methionine residue at position 23 which is cleavable by cyanogens bromide as taught by Ardelt [ ] and the N-terminus amino acid methionine is cleavable with cyanogens bromide, it would have been obvious to one of ordinary skill in the art at the time of the invention to mutate Met-23 to another residue with comparable size such as Leu to cleave the N-terminus Met with cyanogen bromide. The person of ordinary skill in the art would have been guided by the high resolution three dimensional structure of the enzyme taught by Mosimann [ ] to select the most appropriate amino acid that replaces Met-23 without perturbing the structure of onconase. There are many commercially available computer software packages such as QUANTA that utilizes the three dimensional structure of an enzyme/protein to analyze the effects of specific mutation on the structure. Thus, it would have been obvious at the time of invention to one of ordinary skill in the art to chemically synthesize a gene encoding the 104 amino acid sequence of SEQ ID NO: 1 taught by Ardelt [ ] or Mosimann [ ] with the appropriate mutation at position 23 and include the ATG initiation codon (coding for methionine residue in the -1 position) which is required for the expression of almost all proteins in any cell including E. coli (claims 15 and 16). It should be noted that one of ordinary skill in the art would expect that the natural gene coding for the onconase from Rana pipiens oocytes (eukaryotic organism) must contain the ATG codon followed by a codon for glutamine residue as the genetic code does not have a codon for pyroglutamic acid. ATG is the most common initiation codon for protein transcription in both prokaryotic and eukaryotic cells. . . . Once the N-terminus Met is cleaved, the new N-terminus glutamine autocyclizes to form the pyroglutamic acid residue in position 1 as taught by Creighton. The ordinary skill in the art would have had the motivation, skills, knowledge and expectation of success. Thus, the claimed invention was within the ordinary skill in the art to make and use at the time the invention was made and was as a whole, clearly prima facie obvious.

Id. at 10-12.

Appellants argue that the rejection is based on hindsight reconstruction based on their own disclosure, and that at most, the rejection is based on the theory that "obvious-to-try" and arrive at the claimed invention. We agree.

The first issue that we need address in deciding the patentability issues under 35 U.S. C. § 103(a), however, is to determine what is being claimed. See Key Pharmaceuticals v. Hercon Laboratories Corp., 161 F.3d 709, 714, 48 USPQ2d 1911, 1915 (Fed. Cir. 1998). The claims are drawn to an isolated nucleic acid molecule encoding a recombinant ribonuclease from Rana pipiens oocyte comprising a polypeptide of SEQ ID NO: 1, and wherein the amino acid residues present at certain other positions are defined.

We initially note that while the preamble recites that the claim is drawn to an isolated nucleic acid, the body of the claim is drawn to a polypeptide. From the briefs and arguments, it is our understanding that appellants and the examiner are interpreting the claim as requiring an isolated nucleic acid that encodes the polypeptide defined in the body of the claim. Moreover, the claim recites that the polypeptide encoded for by the claimed nucleic acid has the amino acid sequence of SEQ ID NO: 1. While SEQ ID NO: 1 is a polypeptide of defined amino acid sequence, the claim also states that there are certain

positions have amino acids that are not recited in SEQ ID NO: 1. Again, it is our understanding that appellants and the examiner have interpreted the claim to require that what is being claimed is not a nucleic acid encoding a polypeptide of SEQ ID NO: 1, but a nucleic acid encoding a polypeptide, wherein the nucleic

acid has been mutated such that certain defined amino acids of SEQ ID NO: 1 contain an amino acid residue other than that specified by SEQ ID. NO: 1, wherein the changes are particularly defined in the body of the claim.

We can now address the patentability issue under 35 U.S.C. § 103(a) based on the above interpretation of the claim. The burden is on the examiner to make a prima facie case of obviousness, and the examiner may meet this burden by demonstrating that the prior art would lead the ordinary artisan to combine the relevant teachings of the references to arrive at the claimed invention. See In re Fine, 837 F.2d 1071, 1074, 5 USPQ2d 1596, 1598-99 (Fed. Cir. 1988). The findings of fact underlying the obviousness rejection, as well as the conclusions of law, must be made in accordance with the Administrative Procedure Act, 5 U.S.C. 706 (A), (E) (1994). See Zurko v. Dickinson, 527 U.S. 150, 158, 119 S.Ct. 1816, 1821, 50 USPQ2d 1930, 1934 (1999). Findings of fact underlying the obviousness rejection, upon review by the Court of Appeals for the Federal Circuit, must be supported by substantial evidence within the record. See In re Gartside, 203 F.3d 1305, 1315, 53 USPQ2d 1769, 1775 (Fed. Cir. 2000). In addition, in order for meaningful

appellate review to occur, the examiner must present a full and reasoned explanation of the rejection. See, e.g., In re Lee, 277 F.3d 1338, 1342, 61 USPQ2d 1430, 1432 (Fed. Cir. 2002).

In this case, it is hard to determine what is in fact the examiner's position in the statement of the rejection. Appellants argue that the examiner is relying

on an erroneous scientific theory to support the combination, that is, that enzymatic activity is sufficient for cytotoxicity. See Appeal Brief, page 5. The examiner argues, however, that "[t]he roles of the pryroglutamic acid residue in cytotoxicity and enzymatic activities is truly irrelevant," and that "one of ordinary skill in the art would attempt to make a similar protein with similar characteristics regardless of the role or function of the pyroglutamyl residue." Examiner's Answer, page 29. The rejection fails, however, whether it is based on the function of the pyroglutamic acid, or whether it is based on making a similar protein with similar characteristics. We will address both of these rationales in turn.

With respect to the function of the pyroglutamic acid, the rejection asserts that because the pyroglutamic acid residue aids in forming the active site, "any onconase missing [that] important amino acid[ ] is expected to have compromised enzymatic and the associated cytotoxic activity." Thus, the rationale underlying the rejection appears to be that one would be motivated to place the ATG start codon, which encodes methionine, at the -1 position, and also mutate the methionine at position 23 to another amino acid, in order to allow for cyanogen bromide cleavage of the recombinant protein at the methionine at the -1 position, and thus allowing spontaneous cyclization of the glutamine residue at position 1 to the pyroglutamic acid residue. The examiner also argues that the Ardelt reference supports the theory that enzymatic activity is required for cytotoxic activity, and that appellants have not provided any experimental

evidence to counter the teachings of Ardelt. See Examiner's Answer, page 21-22. But, even though Mosimann teaches that the pyroglutamic acid residue is present in the active site, and Ardelt suggests that enzymatic activity may be related to cytotoxicity, the combination demonstrates that, at most, it may have been obvious to try to produce the claimed recombinant protein.

The declarations of Dr. Youle and Dr. Ardelt, see Paper No. 8, establish the unpredictability of the art. The Ardelt declaration states that from the crystal structure as reported by Mosimann, it was not clear what the role of the pyroglutamyl residue in catalysis is, and thus it was not predictable that the pyroglutamyl residue was required for enzymatic and cytotoxic activity. See Ardelt declaration, ¶ 2-5. In addition, Ardelt avers that the examiner has over simplified the issues involved, because the ordinary artisan would have also considered the ability of the enzyme to enter the cell, and its resistance to ribonuclease inhibitor. See Ardelt declaratiion at ¶ 6, Youle declaration at ¶ 3.

Moreover, the Youle declaration also establishes the unpredictability of arriving at the claimed invention by stating that "the synthesis of an active, recombinant form of Onc occurred only after following traditional approaches that failed repeatedly. Finally, after many man years, we unexpectedly were able to produce a cytotoxic recombinant protein by cleaving the N-terminal methionine." Youle declaration, ¶ 2; see also id. at ¶s 6 and 8. The Youle declaration further demonstrates the unpredictability of the problem by declaring that the $rhRNase_{1-11}$-Onc_{10-104} chimera has about 100 times greater catalytic activity than rOnc, yet is much less cytotoxic than Onc. See Youle declaration, ¶ 5.

A determination of obviousness not only requires that the prior art would have suggested the claimed process to one of ordinary skill in the art, but also that the process would have a reasonable likelihood of success when viewed in light of the prior art. See In re Dow Chemical Co., 837 F.2d 469, 473, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988). A rejection based on a reference or a combination of references amounts to an "invitation to experiment," and is thus "obvious-to-try," "when a general disclosure may pique the scientist's curiosity, such that further investigation might be done as a result of the disclosure, but the disclosure itself does not contain a sufficient teaching of how to obtain the desired result, or that the claimed result would be obtained if certain directions were pursued." In re Eli Lilly & Co., 902 F.2d 943, 945, 14 USPQ2d 1741, 1743

(Fed. Cir. 1990). The instant rejection does not contain a sufficient teaching that would allow one to arrive at the instantly claimed polypeptide with a reasonable expectation of success, given the unpredictability and uncertainty in the art as set forth in the Ardelt and Youle declarations.

The second rationale set forth by the examiner in his response to arguments, i.e., that one of ordinary skill in the art would be motivated to make a recombinant protein with similar characteristics to that of the native protein also fails. If the examiner is not relying on the presence of the pyroglutamyl residue in the active site of the protein, no motivation has been set forth by the rejection as to why one of ordinary skill in the art would seek to change the methionine at position 23 rather than the glutamine at position 1. The examiner cites Louis in the rejection as an example of the state of the art. Louis teaches the production of recombinant HIV-1 protease, wherein the HIV-1 is expressed as a fusion protein. In order to liberate the protease from the fusion protein, a linker of ten amino acids was added, wherein the linker is cleavable by the protease. See Examiner's Answer, page 10. Thus, one of ordinary skill could have also expressed the claimed rONC protein as a fusion protein, which obviates the need for a start codon, and also does not require mutation of the nucleic acid encoding the native protein at the methionine at position 23, as cyanogens bromide would no longer be required for use as the cleaving agent. The

rejection therefore sets forth no teaching, suggestion, or motivation, of why the ordinary artisan would have chosen to mutate the methionine at position 23, add the methionine start codon at position -1, and cleave the protein using cyanogens bromide, over other ways of recombinantly expressing the protein, such as that taught by Louis, as each requires a change to the sequence of the native protein.

REVERSED

Sherman D. Winters	)
Administrative Patent Judge	)
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	) BOARD OF PATENT
William F. Smith	)
Administrative Patent Judge	) APPEALS AND
	)
	) INTERFERENCES
Lora M Green	)
Administrative Patent Judge	)

LG/dym

Klarkquist Sparkman Campbell Leigh & Whinston
Whinston
One World Trade Center
121 S W Salmon Street Suite 1600
Portland, OR 97204